

## Restriction Digest Protocol

Note: Restriction Digests should be done on ice.

A Qubit Quantification must be done prior to performing the digest.

- 1) Calculate the amount of DNA you will need to but put into a 50mL reaction.
  - a.  $(\text{Concentration 1})(\text{Volume 1}) = .5\mu\text{g}$ 
    - i.  $\text{Concentration 1} = \text{DNA Concentration}$
- 2) Calculate how much DepC water is necessary to bring the volume (with buffer, enzyme, and DNA) to bring the reaction volume up to 25 $\mu\text{L}$ .
- 3) Appropriately label PCR tubes.
- 4) Add the calculated amount of DepC water into each tubes.
- 5) Add 5 $\mu\text{L}$  of Buffer.
  - a. Note: Check on the NEB website to see which buffer should be used for each enzyme.
- 6) Add 1 $\mu\text{L}$  of each enzyme into each tube.
- 7) Add the calculated amount of DNA into each tube.
- 8) Put tubes into the Thermal Cycler
  - a. Check the NEB website to check the Incubation Temperature. These are enzyme dependent.
  - b. Edit the **iGEMP002** as necessary.
- 9) Store at -20°C